**Supplementary material**

**Design and Rationale of MYOFLAME-19 RCT: MYOcardial protection to reduce PostCOVID inFLAMmatory heart disease due to using CMR Endpoints**

**Running title:**

**MYOFLAME-19: MYOcardial protection to reduce inFLAMmatory heart disease due to COVID-19 Infection using CMR Endpoints**

The study registration identifiers include EudraCT 2022-001682-12; NCT05619653.

**METHODS**

**Study Overview**

|  |  |
| --- | --- |
| **Protocol title :** | **Randomised placebo controlled clinical trial of efficacy of MYOcardial protection in patients with postacute inFLAMmatory cardiac involvEment due to COVID-19 (MYOFLAME-19)** |
| **Sponsor :** | Johann Wolfgang Goethe University Frankfurt |
| **Project phase :** | III |
| **Indication/Diagnosis:** | Inflammatory cardiovascular involvement due to COVID-19, defined by CMR |
| **Rationale**  | Postacute sequelae of COVID-19 infection (PASC) are increasingly recognised complications and are defined by lingering symptoms, not present prior to the infection, typically persisting for more than 4 weeks(1). Cardiac symptoms due to postacute inflammatory cardiac involvement affect a broad segment of people, who were previously well and may have had only mild acute illness (PASC-cardiovascular syndrome, PASC-CVS). Symptoms may be contiguous with the acute illness, however, more commonly they occur after a delay. Symptoms related to the cardiovascular system include exertional dyspnoea, exercise intolerance chest tightness, pulling or burning chest pain, and palpitations. Phenotypically, it is characterised by chronic perivascular and myopericardial inflammation. Cardiac symptoms may be accompanied by manifestations of other organ systems, including fatigue, brain fog, myalgias, skin and joint manifestations, etc, now commonly referred to as the Long COVID or PASC syndrome(2).Evidence suggests inflammatory autoimmune mechanisms, which develop de novo in response to the infection3. In PASC-CVS, early subtle inflammatory heart changes with mild functional impairment are often undetectable by routine diagnostic tests, nor accompanied by significant rise in troponin(1). Studies using CMR imaging have identified changes consistent with non-ischaemic cardiovascular inflammatory involvement22–27, including increased myocardial mapping values and perimyocardial late gadolinium enhancement. Participants may also have subnormal LVEF, however, structural heart disease by profoundly reduced LVEF or dilated heart cavities, or necrotic or thromboembolic complications are not typical findings(3). Likewise, areas of substantial necrosis typically found in classical viral myocarditis(4,5), are rare. These abnormalities can be detected as early as 2 weeks after the infection(6,7) and can be observed several months after the infection(8). Early intervention with immunosuppression and antiremodelling therapy may reduce symptoms and myocardial impairment, by minimising the disease activity and inducing disease remission. Low dose maintenance therapy may help to maintain the disease activity at the lowest possible level. Clinical trials of immunosuppression in participants with viral myocarditis in advanced stages of heart failure have not shown an improved outcome, however there was an improvement of LVEF with antiremodelling therapy in participants with reduced function (9–12). The benefits of early initiations of antiremodelling therapy to reduce symptoms of exercise intolerance are well recognised(13–15), but not commonly employed outside the contexts of heart failure(16) or hypertension. As most participants with inflammatory heart disease only have mild and nonspecific symptoms and few or no structural abnormalities, they are left untreated (standard of care). The aimof this study is to examine the efficacy of a combined immunosuppressive/antiremodelling therapy in participants with PASC symptoms and inflammatory cardiac involvement determined by CMR, to reduce the symptoms and inflammatory myocardial injury and thereby stop the progression to reduced LVEF, HF and death.  |

**Inclusion and exclusion criteria**

|  |  |
| --- | --- |
| **Key Inclusion Criteria:** | * Participants ≥ 18 years
* Participants with documented recent COVID19 infection (>4 weeks)
* PASC Syndrome, defined by persistence or new symptoms, not present prior to the infection.
* CMR evidence of inflammatory cardiac involvement at BL by any of the following criteria:
	+ Increased native T1≥ 1130 ms at 3.0 Tesla (or 1030 ms at 1.5 Tesla) and/or;
	+ Increased native T2 ≥39.5 ms at 3.0 Tesla (or 49.5 at 1.5 Tesla) and/or
	+ present non-ischaemic myopericardial LGE and/or;
	+ LVEF ≥45 - ≤50%.
* Willingness to comply with the study procedures and study protocol
 |
| **Key Exclusion Criteria** | * Severe acute COVID illness requiring hospitalisation
* Known allergy to or intolerance of the study medications
* Symptomatic hypotension (systolic blood pressure less than 90 mm Hg), not reversible with oral hydration
* Any previous or current use of ACE inhibitors, AR Blockers
* Any previous oral prednisolone, or any other immunosuppressive or biological treatment (within prior 10 weeks)
* History or CMR evidence of pre-existing significant heart disease, including:
	1. Known cardiac impairment with LVEF ≤44%
	2. Congestive heart failure (NYHA III-IV)
	3. Active heart failure treatment
	4. Established ischaemic heart disease, peripheral arterial disease and/or cerebrovascular disease;
	5. Persistent or permanent atrial fibrillation or significant heart rhythm abnormalities
	6. Congenital or clinically relevant valvular heart disease (moderate or severe)
	7. Specific cardiomyopathy (hypertrophic, hypertensive heart disease, amyloidosis, previous myocarditis, non-ischaemic dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, non-compaction cardiomyopathy, etc).
* Known significant concomitant diseases that are likely to interfere with the evaluation of the participant’s safety and of the study outcome (e.g. diabetes, lung or hepatic disease, epilepsy, psychiatric disorders, renal disease with a current estimated GFR <30 mL/min/1.73 m² using MDRD formula, chronic systemic infection or immunocompromise)
* Exceeding scanner bore and table-holding capacity: Weight >125 kg, BMI > 35 kg/m2
* Contraindications to contrast-enhanced CMR imaging, e.g.
1. MR-unsafe implantable device
2. known allergy to gadolinium-based contrast agent (CBGA)
* For female participants:
	1. Pregnant or breastfeeding women
	2. Women of childbearing potential not willing to use highly effective contraception (as defined in 18.2.13)
* Known alcohol, drug or chemical abuse
* Participants currently participating in an investigational study or for whom participation is planned.
* Unable to provide written informed consent.
* Participants with CMR evidence of structural heart disease or incidental heart rhythm abnormalities will be advised to see their own doctor for further investigation.
 |

**Investigational medicinal products**

The IMPs in this study are low dose Prednisolone and Losartan, or their respective placebos. Investigators and study participants are blinded to the underlying group allocation. The study medication is administered orally according to the treatment schedule, irrespective of the treatment group. Schedules of Prednisolone taper and Losartan dose escalation are foreseen for treatment and placebo arm, details are provided in Supplementary material.

### Active Study Medication

|  |  |
| --- | --- |
| **Active substance** | **Losartan** |
| Dosage form | Capsules à 12.5 mg and 25 mg |
| Starting dose | 12.5 mg once a day |
| Taper regime | gradual increase in doses of 12.5mg every 2 weeks maximally tolerated dose |
| Maintenance dose | Maximal 50 mg |
| Duration of treatment | Maximum of 16 weeks |
| Mode of application | Oral |
| Time of application  | Evening (0-0-1), or in divided doses (1-0-1) |
| Storage | Storage in a dry place at room temperature (≤ 25 °C) |

Guidance – as tolerated: Gradual increase by 12.5 mg every 2 weeks (week 1-2: 12,5 mg, week 3-4: 25 mg, week 5-6: 37.5 mg, week 7-16: 50 mg (maintenance dose).

|  |  |
| --- | --- |
| **Active substance** | **Prednisolone** |
| Dosage form | Capsules à 5 mg and á 10 mg |
| Starting dose | 20 mg once a day |
| Taper regime | Taper by 5 mg every two weeks |
| Maintenance dose | 5 mg |
| Duration of treatment | Maximum of 16 weeks (1-0-0) |
| Mode of application | Oral |
| Time of application  | Morning dose (1-0-0) |
| Storage | Storage in a dry place at room temperature (≤ 25 °C) |

Guidance – as tolerated: Fixed taper by 5 mg every 2 weeks (week 1-2: 20 mg, week 3-4: 15 mg, week 5-6: 10 mg, week 7-16: 5 mg (maintenance dose).

### Placebo

The placebo contains all of the inactive ingredients and none of the active ones. Placebo will match active study drug in smell, taste, colour and appearance to assure proper blinding.

Matching Capsules of Losartan and Prednisolone.

|  |  |
| --- | --- |
| **Substance** | **Placebo 1**  |
| Dosage form | Look-alike Capsules à 12.5 mg and 25 mg |
| Starting dose | 12.5 mg once a day |
| Taper regime | gradual increase by 12.5 mg to maximally tolerated dose every 2 weeks |
| Maintenance dose | Maximal 50 mg |
| Duration of treatment | Maximum of 16 weeks |
| Mode of application | Oral |
| Time of application  | Evening (0-0-1), or in divided doses (1-0-1) |
| Storage |  Storage in a dry place at room temperature (≤ 25 °C) |

|  |  |
| --- | --- |
| **Substance** | **Placebo 2** |
| Dosage form | Look-alike Capsules à 5 mg and á 10 mg |
| Starting dose | 20 mg once a day |
| Taper regime | Taper by 5 mg every two weeks |
| Maintenance dose | 5 mg |
| Duration of treatment | Maximum of 16 weeks (1-0-0) |
| Mode of application | Oral |
| Time of application  | Morning dose (1-0-0) |
| Storage | Storage in a dry place at room temperature (≤ 25 °C) |

**Table 1.** Schedule of study related procedures.

|  | Activities/Examinations | Baseline | Study Period (Treatment) | Follow Up |
| --- | --- | --- | --- | --- |
|  | Weeks | **BL** | **W0§** | W2§ | **W6** | W12§ | **W16** | FU-Y1# |
|  | Visit window | -6W-0D |  | +/- 10D | +/- 14D | +/- 14D |
|  | Visits | 0 | **1** | 2 | **3** | 4 | **6** | 6 |
| Clinical assessment | Informed Consent (written) | **X** |  |  |  |  |  |  |
| Inclusion/Exclusion criteria | X |  |  |  |  |  |  |
| Demographic data and medical history | X |  |  |  |  |  |  |
| Concomitant Medication | X |  | X | **X** | X | **X** |  |
| Symptoms Scores Questionnaires | X |  | X | **X** | X | **X** |  |
| QoL Questionnaires | X |  |  | **X** |  | **X** |  |
| Vital signs | X | X\*\*\* | X\*\*\* | **X** | X\*\*\* | **X** |  |
| Outcome Endpoints  |  |  |  |  |  |  | X |
| Laboratory | \*FBC, blood chemistry, liver function tests (only at BL), CRP, troponin, NTproBNP, D-dimer, Fibrinogen | **X** |  |  | **X** |  | **X** |  |
| Lipid profile, HbA1c, thyroid function tests | **X** |  |  |  |  | **X** |  |
| Blood samples stored (-80°C) | **X** |  |  | **X** |  | **X** |  |
| \*\*Pregnancy test\* | **X** |  |  |  |  | **X** |  |
| ECG | 12 lead ECG ((intervals (ms): PQ; QRS, QT) | **X** |  |  | **X** |  | **X** |  |
| CMR | Myocardial mapping | **X** |  |  |  |  | **X** |  |
| Strain & Ejection fraction | **X** |  |  |  |  | **X** |  |
| LV volume and mass | **X** |  |  |  |  | **X** |  |
| Aortic wall imaging (LGE) and stiffness (PWV) | **X** |  |  |  |  | **X** |  |
| Myocardial LGE | **X** |  |  |  |  | **X** |  |
| CPET | achieved Work Rate, VO2max, VCO2 max, RER, AT, slope | **X** |  |  |  |  | **X** |  |
| Participants´ diaries | \*\*\*Home-based BP and HR measurements |  | X\*\*\* |  |  |  |  |  |
| IMP diary |  | **X** | X | **X** | X | **X** |  |
|  | Provision of IMP to randomized participants | **X** |  |  |  |  |  |  |
|  | Compliance |  |  | X | **X** | X | **X** |  |
|  | Adverse Events |  |  |  |  |  |  |  |

\*The processing of laboratory analyses will go ahead for participants fulfilling CMR criteria only

\*\*all female participants at baseline and W16. In addition, women of childbearing potential (WOCBP) will be instructed to contact their study physician immediately in the absence of menstruation or in case of other clinical evidence of pregnancy for further clarification. Please also refer to Section 9.4.4 Pregnancy.

\*\*\* home-based measurement of blood pressure/heart rate

§will be performed as a telephone call or video evaluation (may be performed as remote visit at W6 in case of long-distance travelling).

#Follow-ups 1Y: Proportion of participant with HF or MACE after 1 years; 1- year Event-free survival. Please refer to Section 9.2.11 (Outcome Endpoints) for details on collection and analysis of 1-Year outcomes.

**Assessments**

Standardised questionnaires

**Long COVID Questionnaire (modified from Sudre et al)**[1]**, in German.**

Bestehen bei Ihnen die folgenden Beschwerden:

FA Fatigue (Fatigue) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

PEM Post-exertional Malaise\* 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

HA Headache (Kopfschmerzen) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

POTS Excessive Tachycardia, Herzrasen (POTS) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

SOB Shortness of breath (Kurzatmigkeit) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

LOS Loss of smell (Geruchsverlust) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

PC Persistent cough (Hustenreiz) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

ST Sore throat (Halsschmerzen) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

FV Fever (Fieber) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

UMP Unusual muscle pains (Muskel/Gliederschmerzen) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

SM Skipped meals (Übersprungene Mahlzeiten) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

CP Chest Pain (Brustschmerzen) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt) When yes - > Modified CCS

DI Diarrhoea (Durchfall) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

HV Hoarse Voice (Heisere Stimme) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

AP Abdominal Pain (Bauchschmerzen) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

DE Brain Fog (Brain Fog) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

TACHY – Excessive Tachycardia (‘POTS’) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt)

LOC Loss of Consciousness (Ohnmacht) 0-no (nein), 1-yes (Ja), 99-unknown (unbekannt) When yes:

-> Near faint (Nahezu ohnmächtig)

-> Syncope (Synkope)

- > Reanimation

\*Post-Exertional Malaise (PEM) ist die Verschlechterung der Symptome nach selbst geringer körperlicher oder geistiger Anstrengung, wobei sich die Symptome typischerweise 12 bis 48 Stunden nach der Aktivität verschlimmern und Tage oder sogar Wochen anhalten.

Post-Exertional Malaise (PEM) is the worsening of symptoms after even mild physical or mental exertion, with symptoms typically worsening 12 to 48 hours after activity and lasting for days or even weeks.

**Modified Chest Discomfort Severity Score**

Chest discomfort was graded using a modified Chest Discomfort Scale based on the Canadian Chest Pain Scale[2], which was not limited to the typical anginal-type of chest pain, but also included, deep, dull, pulling, burning or sharp chest discomfort or tightness, radiating into neck, back, shoulders or arms. To differentiate this from precordial catch symptoms and abdominal stiches, the criterion for chest pain was if lasting for more than 10 minutes.

|  |  |
| --- | --- |
| The original Chest Pain Scale | Description of Symptoms (Beschreibung der Beschwerden) |
| 0 | No symptoms (Keine Beschwerden) |
| I | Presence of chest discomfort during strenuous, rapid, or prolonged ordinary activity (walking or climbing the stairs).(Vorhandensein von Brustbeschwerden während anstrengender, schneller oder längerer normaler Aktivität (Gehen oder Treppensteigen). |
| II | Presence of chest discomfort during or after ordinary activities, when they are performed rapidly, or by change of position, under emotional stress, but also walking uphill, climbing more than one flight of ordinary stairs at a normal pace and under normal conditions.(Vorhandensein von Brustbeschwerden während oder nach gewöhnlichen Aktivitäten, wenn sie schnell ausgeführt werden, oder durch Positionswechsel, unter emotionalem Stress, aber auch beim Bergaufgehen, beim Steigen von mehr als einer gewöhnlichen Treppe in normalem Tempo und unter normalen Bedingungen.) |
| III | Presence of chest discomfort during or after activities of daily life at normal pace and conditions. (Vorhandensein von Brustbeschwerden während oder nach Aktivitäten des täglichen Lebens bei normalem Tempo und normalen Bedingungen.) |
| IV | No exertion needed to trigger chest pain, present at rest, recurring, or present at all times. (Keine Anstrengung erforderlich, um Brustschmerzen auszulösen, die in Ruhe vorhanden, wiederkehrend oder jederzeit vorhanden sind.) |

**MRC Dyspnoea Severity Score**

Dyspnoea was graded using modified Medical Research Council Dyspnea[3].

|  |  |
| --- | --- |
| MRC Dyspnoea Scale | Description of Symptoms (Beschreibung der Beschwerden) |
| 0 | No symptoms (Keine Beschwerden) |
| 1 | Breathless with strenuous exercise (Atemlos bei anstrengender Belastung) |
| 2 | Short of breath when hurrying on the level or walking up a slight hill (Kurzatmigkeit, wenn man auf der Ebene eilt oder einen leichten Hügel hinaufgeht) |
| 3 | Walks slower than people of the same age on the level or stops for breath while walking at own pace on the level (Es geht langsamer als Gleichaltrige auf der Ebene oder hält an, um Luft zu holen, während er in seinem eigenen Tempo auf der Ebene geht) |
| 4 | Stops for breath after walking 100m (Atempause nach 100m Gehen) |
| 5 | Too breathless to leave the house or breathless when dressing (Zu atemlos, um das Haus zu verlassen oder atemlos beim Anziehen) |

### RAND 36-item Health Survey, Version 2.

This Quality-of-Life Survey will be completed on paper for BL and W16 by the participant and transferred to the eCRF by the site staff

https://www.rand.org/health-care/surveys\_tools/mos/36-item-short-form.html

### Participant Blood Pressure and Heart Rate Diary and Participant Medication Diary

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Date | Time | Systolic Blood pressure (mmHg) | Diastolic Blood Pressure (mmHg) | Heart rate (bpm) | Prednisolone (dose, mg) | Losartan (dose, mg) |
| W1-W6 | AM |  |  |  |  |  |
| PM |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| From W7-10, 3 times a week, or when feeling unwell | AM |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**Data Analyses**

**Primary endpoint analysis** will examine the absolute LVEF change to baseline at W16, measured by CMR, compared between the verum and placebo group by absolute treatment difference. Superiority of the verum group to improve LVEF compared to placebo will be tested as H0: μVERUM = μPL vs. H1: μVERUM ≠ μPL (see section (11.1) at the 5% significance level in a two-sided manner by an unpaired t-test.

**Secondary endpoints analyses**

For all secondary endpoints, descriptive statistics will be used to compare baseline characteristics between the two groups. Continuous variables will be expressed as mean ± SD, categorical variables will be expressed in counts with percentages. The differences in CMR parameters and other continuous secondary outcomes from baseline to 16 weeks post randomization between the two treatment groups will be analysed using an ANCOVA. Treatment effect estimates and 95% confidence intervals will be determined. These hypotheses tests will be of explorative nature and tested at a global 5% significance level between treatment groups/factors in a 2-sided manner. Correlations between secondary endpoints may be assessed using contingency tables (relative risk, odds ratio), correlation coefficient (Pearson, Spearman, Kendall’s Tau) or, depending on the nature of the correlation. A treatment comparison of the occurrence of frequent AEs and prespecified outcome endpoints will be performed using a Chi Square Test (or exact Fisher Test in case of small abs. frequencies) with α=5%. Analysis methods may employ Kaplan-Maier curves, Log-rank tests, lifetable analysis, or Cox-regressions. A competing risk analysis will then also be considered.

**Compliance and tolerance**

Compliance will be assessed by the patient diary and drug accountability (e.g. empty Blisters) for IMP intake. Summary statistics will be given for the treatment intake (IMP), i.e. consumed medication prescribed, stratified by treatment and visits. Relevant information of the patient diary will be summarized and reported descriptively or as documented.

Tolerance will be assessed by the absolute and relative (%) frequency of patients changing their treatment number of patients required dose reduction or treatment cessation due to side effects. Cumulative oral corticosteroid dose will be calculated and described by summary statistics at all visits and illustrated by mean +/- SD or boxplots over time stratified by treatment group.

**Objectives & Endpoints:**

|  |  |
| --- | --- |
| Primary efficacy objective:  | To determine efficacy of a combined immunosuppressive and antiremodelling therapy in COVID-19 related postacute inflammatory cardiovascular involvement determined by CMR to reduce inflammatory myocardial injury compared to placebo  |
| Primary efficacy endpoint: | Absolute LVEF change to baseline at W16, measured by CMR, compared between the verum and placebo group by absolute treatment difference  |
| Secondary efficacy objectives: | To determine the efficacy of a combined immunosuppressive and antiremodelling therapy for 16W in COVID-19 related postacute inflammatory cardiovascular involvement determined by CMR compared to placebo at all available time points compared to BL, by improvement in other clinical parameters.1. Scar burden by late gadolinium enhancement (LGE)
2. Cardiopulmonary exercise testing (CPET)
3. Myocardial T1 and T2 mapping measures
4. Cardiac structure (LV volume and mass)
5. Myocardial deformation/strain
6. Aortic wall imaging (LGE) and stiffness (PWV)
7. Symptom Score (Modified Canadian Chest pain scale, NYHA, MRC Dyspnoea scale, Long COVID Questionnaire(2))
8. QoL (RAND 36-Item Health Survey Version 2.0)
9. Compliance/Tolerance of therapy
10. Assessment of treatment response
11. Progression to HF, MACE and death, compared to placebo after 1- and years’ time.
 |
| Secondary efficacy endpoints: | Secondary endpoints will be analysed at all available visits for both treatment groups. For all continuous endpoints, “changes” refer to the difference between the visit measurement and baseline (BL, absolute and in %):* Mean LGE extent (%) and change thereof compared to BL
* CPET (achieved Work Rate, VO2max, VCO2 max, RER, AT and slope) and change thereof compared to BL
* Mean T1 and T2 values (ms) and change thereof compared to BL
* Mean LV volume (ml/m2) and mass (g/m2) and change thereof compared to BL
* Mean Myocardial strain (%) and change thereof compared to BL
* Aortic wall thickness (LGE, mm) and change thereof compared to BL;
* Mean Pulse wave velocity (m/s) and change thereof compared to BL;
* Average Symptom Score and change thereof compared to BL;
* Compliance: Frequency of prescribed medication consumed, participant diary and drug adherence, total cumulative steroid dose;
* Tolerance: number of participants who required dose reduction or treatment cessation due to side effects, especially due to
	+ Hypotension

Unblinding due to safety issues * Number of Responders by achieving:
	+ partial response: a normal CMR result is defined as normal T1 and T2, normal gender-age predicted LVEF, non-dilated LV
	+ total response: in addition to the above absence of LGE
* Proportion of participant with HF or MACE after 1 years
* 1- and year Event-free survival
 |
| **Key safety parameters:** | Frequency, severity and number of adverse events (AE):* Proportion of participants with infectious complications (a combination of at least two of the following:
	+ fever ≥38.5°C,
	+ rise on hsCRP,
	+ neutrophilia,
	+ lymphocytosis,
	+ need for antiviral or antibiotic treatment)
* Proportion of participants with symptomatic hypotension (blackouts and systolic BP<90 mmHg) accompanied by a syncope
* Proportion of participants with symptomatic tachycardia with heartrate >110/min accompanied by a syncope;
* Proportion of participants with a significant rise in cardiac biomarkers (hsTNT, NTproBNP, >3-times the BL)
* Proportion of participants with onset of clinical hear failure.
* Absolute changes in lipid profile, HbA1c, thyroid function tests compared to BL
* Proportion of participants with a significant drop in eGFR compared to BL (>25%)
* Proportion of participants with worsening of cardiovascular symptoms (increase in CCS, NYHA class, clinical heart failure)
* Proportion of participants with acute psychotic episode
* Proportion of participants with hypertensive crisis (with systolic BP>180mmHg and diastolic >120 mmHg)
 |
| **Additional****Assessments:** | Blood samples (whole blood, serum and plasma) will be retained for measurement for future measurements, as indicated in the section 9.2.6. |

**Data analysis**

All primary and secondary efficacy analysis will be performed for the Intention-to-treat (ITT) and for the Per Protocol (PP) population (definitions in ***supplementary material***). All safety analysis will be performed for the safety set. ITT analysis will apply the analysis strategy of the treatment policy, while PP analysis will apply while on treatment strategy. All primary and secondary endpoints will also be analysed in a descriptive manner using summary statistics stratified by treatment and visit. The mean (or median), respectively proportion, of the treatment difference in selected endpoints will be reported at all visits of interest together with the 95% confidence interval (or IQR). All named analyses will be performed on patient level. Detailed data analyses and sample size considerations are provided in supplementary material.

**Primary endpoint analysis** will examine the absolute LVEF change to baseline at W16, measured by CMR, compared between the verum and placebo group by absolute treatment difference. Superiority of the verum group to improve LVEF compared to placebo will be tested as H0: μVERUM = μPL vs. H1: μVERUM ≠ μPL (see section (11.1) at the 5% significance level in a two-sided manner by an unpaired t-test.

**Secondary endpoints analyses**

For all secondary endpoints, descriptive statistics will be used to compare baseline characteristics between the two groups. Continuous variables will be expressed as mean ± SD, categorical variables will be expressed in counts with percentages. The differences in CMR parameters and other continuous secondary outcomes from baseline to 16 weeks post randomization between the two treatment groups will be analysed using an ANCOVA. Treatment effect estimates and 95% confidence intervals will be determined. These hypotheses tests will be of explorative nature and tested at a global 5% significance level between treatment groups/factors in a 2-sided manner. Correlations between secondary endpoints may be assessed using contingency tables (relative risk, odds ratio), correlation coefficient (Pearson, Spearman, Kendall’s Tau) or, depending on the nature of the correlation. A treatment comparison of the occurrence of frequent AEs and prespecified outcome endpoints will be performed using a Chi Square Test (or exact Fisher Test in case of small abs. frequencies) with α=5%. Analysis methods may employ Kaplan-Maier curves, Log-rank tests, lifetable analysis, or Cox-regressions. A competing risk analysis will then also be considered.

**Compliance and tolerance**

Compliance will be assessed by the patient diary and drug accountability (e.g. empty Blisters) for IMP intake. Summary statistics will be given for the treatment intake (IMP), i.e. consumed medication prescribed, stratified by treatment and visits. Relevant information of the patient diary will be summarized and reported descriptively or as documented.

Tolerance will be assessed by the absolute and relative (%) frequency of patients changing their treatment number of patients required dose reduction or treatment cessation due to side effects. Cumulative oral corticosteroid dose will be calculated and described by summary statistics at all visits and illustrated by mean +/- SD or boxplots over time stratified by treatment group.

No interim analyses are planned in this trial.

**Criteria for evaluation of Study results**

**Criteria for evaluation of Efficacy**

**Primary Efficacy Endpoint**

Absolute LVEF change to baseline at W16, measured by CMR, compared between the verum and placebo group by absolute treatment difference.

**Secondary efficacy endpoints**

The secondary efficacy parameters are listed below.

The secondary endpoints include the following continuous endpoints, where “changes” refer to the difference between baseline and W16 (BL, absolute and in %):

* Mean LGE extent (%) and change thereof compared to BL
* CPET (achieved Work Rate, VO2max, VCO2 max, RER, AT, slope) and change thereof compared to BL
* Mean T1 and T2 values (ms) and change thereof compared to BL
* Mean LV and RV volumes (ml/m2) and LV mass (g/m2) as well as derived parameters and change thereof compared to BL
* Mean Myocardial strain (%) and change thereof compared to BL
* Mean Pulse wave velocity (m/s) and change thereof compared to BL
* Aortic wall thickness (LGE, mm); and change thereof compared to BL
* Average Symptom Score and change thereof compared to BL
* Compliance: Frequency of prescribed medication consumed, patient diary and drug adherence, total cumulative steroid dose;
* Tolerance: number of patients who required dose reduction or treatment cessation due to side effects, especially due to
	+ Hypotension
	+ Unblinding due to emergency safety issues
* Number of Responders by achieving:
	+ partial response: a normal CMR result is defined as normal T1 and T2, normal gender-age predicted LVEF, non-dilated LV
	+ total response: in addition to the above absence of LGE
* Proportion of patient with HF or MACE after 1 year
* 1- year Event-free survival

The results of the findings for secondary endpoints are supportive and not confirmatory on their own. Therefore, the effect of the randomly allocated study treatment on the secondary endpoints are reported, in addition to measures of effect sizes, standard errors, confidence intervals, with nominal p values, unadjusted for testing multiplicity.

**Criteria for Evaluation of Safety**

The primary safety objective is to demonstrate that a combined immunosuppressive and antiremodelling therapy in proposed doses in this patient population is safe.

**Safety Parameters**

Safety parameters include the number or frequency, severity, and number of adverse events (AE), serious AE (SAEs), defined by:

* Proportion of patients with serious infectious complications (fever ≥38.5°C, accompanied by rise on hsCRP, neutrophilia, lymphocytosis, septic shock, need for antiviral or antibiotic treatment)
* Proportion of patients with symptomatic hypotension (dizziness, blackouts, and systolic BP<90 mmHg) or bradycardia (heart rate <40/min)
* Proportion of patients with a significant rise in cardiac biomarkers (hsTNT, NTproBNP, >3-times the BL)
* Proportion of patients with a significant drop in eGFR compared to BL (>25%) compared to BL
* Proportion of patients developing hypertensive crisis

**Description of Population and Patient Groups for Analyses**

Patients with long COVID19 with evidence of cardiac involvement by CMR criteria and no known previous cardiovascular disease, who fulfil the inclusion and not fulfil the exclusion criteria. Patients will be randomised into verum and placebo groups.

**Full Analysis Set (FAS)**

The full analysis set (FAS) is defined to include all participating patients who were randomized into the study.

**Intention to treat set (mITT)**

The primary analysis will be performed on the ITT population. All patients who were randomized will be included in the ITT analysis. The intention to treat set is defined as the subgroup of patients of the FAS who had two assessments with CMR (BL and follow-up) and who received at least 4 weeks of treatment according to their treatment arm after study inclusion as eligible for data analysis. This set will be basis for the primary analysis. Patients excluded from statistical analysis will be listed by reason in concordance with ICH E3.

**Per Protocol Population**

Per protocol population (PP) is defined to include all patients who have another CMR at 16 weeks and have complied 75% with the study drug regimen will be included in the per-protocol analysis if the data confirms its feasibility and validity. Specific reasons for warranting exclusion will be documented prior to the closing of the database. Not all protocol deviators and violators will be excluded from the per protocol population.

**The safety analysis set** is defined as all patients who received at least one dose of IMP treatment (verum or placebo) during the study. Safety data will be analysed describing frequency, severity and types of adverse events for all treatment groups. The proportion of AEs (including AESI and special situations) related to the contrast agent will also be analysed. All adverse events will be coded and tabulated by system organ class and preferred term for individual events within each system organ class and will be presented in descending frequency. Adverse events will also be tabulated by severity and relationship to the study medication. Serious adverse events will be summarized separately. Listings will be produced in concordance with ICH E3, including actions taken and outcome.

**Statistical considerations**

**Sample size**

We consider a treatment difference in mean LVEF change of 2-4% with SD=9%, and a 1:1

treatment allocation, corresponding to an effect size of Cohen’s d=0.22 to 0.44, i.e. a small to

moderate effect. Based on preliminary calculations, the unpaired t-test with a two-sided α=5%, a power of 80%, and an assumed Cohen´s effect size of 0.35 leads to 130 patients per study arm (260 patients for both arms). Assuming a drop-out rate of about 8% following randomization, a total of 280 patients will have to be randomized. A SCR failure rate of 50% will be expected (presence of cardiovascular abnormalities in appr. 25% of patients and no detectable abnormalities in 25%). That means that approx. 560 to 600 patients will be expected to be screened.

**Justification of primary endpoint**

The aim of this study is to allow the comparison of changes in LVEF, measured by CMR, between the study arms LVEF is a standard measure of cardiac performance in clinical trials. It can also be translated into other imaging modalities (echocardiography, cardiac CT). Based on the substantial validation and standardisation evidence supporting the accuracy of volumetric analyses (summarised in(107)), CMR is the gold-standard technique for measurement of LVEF. The chosen primary endpoint based on CMR is an objective endpoint and will be derived using highly standardised procedures. The automatization and AI-supported acquisition and postprocessing permit only observers' interference, yielding a reliable and reproducible objective parameter. The superior inter-study reproducibility of CMR based measurements compared with echocardiography allows for considerably lower calculated sample sizes (reductions of 55% to 93%) to show clinically relevant changes in LV dimensions and function. This is irrespective of the hearts structure (normal, dilated or hypertrophied)(117).

**Handling of missing data and values above/below the LOQ**

In general, missing data of a parameter will not be transformed and remains untouched, unless > 5% of the data is missing or defined otherwise. Summary statistics will generally be given including the underlying number of valid individual values and missing data.

For the primary analysis, the unpaired t-test will be applied. If a large number of values are missing, LOCF will be considered for the LVEF to determine the primary endpoint. If data is imputed, primary analysis will be compared between the imputed data and the observed cases.

Values below/above the LOQ will in general be imputed by a suitable fixed value (e.g. LLOQ, ULOQ, Zero), depending on the nature of the laboratory value and its time of assessment. All handling of values above/below the LOQ and handling of missing values will be described in the Statistical Analysis Plan (SAP).

**Handling of therapy changes and unscheduled visits**

Unscheduled visits will not be included in the analysis. Participants who change therapy before W16 will continue to perform all further planned study visits. Data up until the treatment change will be used for Per-Protocol analysis (as a while on treatment strategy for the intercurrent event of a treatment change), whereas the complete dataset will be used in the Intention to Treat (ITT) analysis (Following a treatment policy strategy).

Protocol deviations, drop-outs and participants excluded from analysis All protocol deviations, drop-outs of the study and participants excluded from analysis will be described by summary statistics and presented in a CONSORT. Where possible, they will be categorized by reason. Drop-outs include withdrawals, screening failures and participants who are lost-to-follow-up.

**Replacement Policy (Ensuring Adequate Numbers of Evaluable Subjects)**

Up to a maximum of 20 participants (i.e., appr. 6%) will be replaced due to dropouts and missing values of primary endpoint. Reasons for dropouts have to be documented in the CRF.

**For Centres**

A centre may be replaced for the following administrative reasons: excessively slow recruitment or poor protocol adherence or any other suspected compliance problem with GCP.

**Management of Adverse Events**

**Adverse Events Reporting**

An adverse event (AE) is defined as any untoward medical occurrence or worsening of a preexisting medical condition in a patient or clinical investigation subject administered an

investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Adverse events can be spontaneously reported or elicited during open-ended questioning,

examination, or evaluation of a patient. (In order to prevent reporting bias, patients should not be questioned regarding the specific occurrence of one or more AEs.) Pre-existing conditions, which worsen during a study, are to be reported as Adverse Events.

Any AE that results in any of the following outcomes will be considered a **Serious Adverse Event (SAE)**:

1. Death
2. Life-threatening situation (patient was at risk of death at the time of the event. This does not refer to an event that might have caused death if it was of greater intensity.)
3. New in-patient hospitalization or prolongation of existing index hospitalization
4. Persistent or significant disability or incapacity
5. Congenital anomaly or birth defect
6. Important medical events that may not result in death, be life-threatening, or require hospitalization but may jeopardize the patient and may require medical or surgical intervention to prevent one of the above outcomes (based upon appropriate medical judgment), e.g.,

Intensity of adverse events will be graded on a three-point scale (mild, moderate, severe) and

reported in detail as indicated in the CRF (see W.H.O. Handbook for Reporting Results of Cancer Treatment).

1. Mild: Discomfort noticed but no disruption of normal daily activity.
2. Moderate: Discomfort sufficient to reduce or affect daily activity.
3. Severe: Inability to work or perform normal daily activity

By contrast, the term “serious” is used to describe an event based on an event outcome or actions usually associated with events that pose a threat to a patient’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

For all collected AEs, the clinician who examines and evaluates the patient will determine the

event’s **causality** to the study drugs or contrast agent based on temporal relationship and their clinical judgment. The relationship will be given for both non-serious and serious AEs. The degree of certainty about causality will be graded using the categories below:

- Definitely Related: There is clear evidence to suggest a causal relationship, and other

possible contributing factors can be ruled out.

- Probably Related: There is evidence to suggest a causal relationship, and the influence of

other factors is unlikely.

- Possibly Related: There is some evidence to suggest a causal relationship. However, the

influence of other factors may have contributed to the event.

- Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal

relationship to drug administration makes a causal relationship improbable and in which

other drugs or chemicals or underlying disease provides plausible explanations.

- Not Related: The AE is completely independent of study drug administration, or contrast

agent, and/or evidence exists that the event is definitely related to another aetiology.

Pre-existing conditions should be recorded upon patient enrolment (including start date of the condition, and severity - mild, moderate, severe). After the patient signs the informed consent form, any worsening of these conditions would be recorded.

Any new conditions would be recorded including date of onset, date of resolution, severity (mild, moderate, severe, or serious as defined above) and possible relationship to study drug, or contrast agent. As part of the source notes, follow up clinical assessments, laboratory tests, ECGs and diagnostic imaging related to adverse event should be documented.

Adverse events, especially those for which the relationship to IMP is considered “related” by the investigator, should be followed until resolved or until FU visit. If a clear explanation is established, it should be recorded on the CRF.

**Other adverse events**

The following AEs should be recorded and reported to the Sponsor or Project Management within 24h of knowledge, via eCRF, as described above.

**AEs of special interest:**

Any AE/SAE which is considered related (according to investigator´s assessment) to the contrast agent and which persists for 4 weeks or more post its administration.

**AEs of special situations (for study medication and contrast agent):**

1. reports of misuse, abuse, overdose, medication error and other use outside what is

foreseen in the protocol,

2. drug dependency, withdrawal syndrome,

3. occupational exposure,

4. suspected transmission of an infectious agent,

5. drug interactions

**Handling of Safety Parameters**

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject’s clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, vital signs or findings from other appropriate tests and procedures. All adverse events related to the study therapy, serious adverse events and pregnancies that occur within the AE reporting deadline will be reported to the sponsor.

All clinical AEs encountered during the clinical study will be recorded in medical records and on the AE page of the CRF. All AEs and SAEs will be recorded by the Investigator from the time *of written informed consent till 4 weeks after the last administration of IMP (W16) or till 4 weeks after last administration of gadolinium-based contrast agent, whichever occurs last*. This being considered the AE reporting deadline.

**Serious Adverse Events (Immediately Reportable to the Sponsor or Project Management)**

Any clinical adverse event or abnormal laboratory test value that is serious, irrespective of the treatment received by the patient, must be reported to the sponsor within 24h of knowledge (expedited reporting). Reporting takes place regularly via the eCRF, therefore the contact details below should only be used in emergencies / in the event of technical problems. Safety reporting is carried out in accordance with local legislation and the applicable guidelines.

**Treatment and Follow-up of Adverse Events**

Adverse events, especially those for which the relationship to test "drug" is “related", should be followed up until resolved or stable, when considered related. SAEs should be followed until resolved or stable when considered related. If a clear explanation is established, it should be recorded on the CRF. Treatment of AEs is at the discretion of the investigator and should follow the standards of medical care at the investigator’s institution.

**Premature Withdrawal of the Patient**

Patients may voluntarily withdraw their consent to study participation at any time without giving any reason. The investigator may also, at their discretion, withdraw the patient from participating in this study at any time, or the sponsor may discontinue the study.

Reasons for early withdrawal from the study should be documented in the eCRF as:

- Study closed / terminated

- Patient died or is lost to follow-up

- Investigator’s decision

- Patient withdrew consent to trial participation

For patients who withdraw consent to trial participation, participation ends immediately. Date of withdrawal from the study, with reason for withdrawal (if applicable), will be documented in the patient's medical record and recorded on the eCRF. In the case of death, a death certificate should be obtained, if possible, with the cause of death evaluated and documented.

**Criteria for Termination of the Study**

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the sponsor and the investigator will assure that adequate consideration is given to the protection of the patient’s interests.

Following criteria could lead to a discontinuation or early termination of the study:

* Patients’ safety
* Negative benefit / risk assessment due to new information
* Recruitment or procedural issues.

In case of premature termination of the study, all collected data will be analysed and a report has to be written. The sponsor must inform the competent authority, federal regulatory authority, the ethics committees and other authorities of member states of the European Union where the study is conducted within 15 days, giving detailed reason for the premature termination.

**Plan for Treatment after the End of Study Treatment**

The investigator will inform the patients about available treatments for after the

completion of the study and the decision will be left to the patient, general practitioner and investigator’s discretion.

**Pregnancy and contraception**

**Women of childbearing potential (WOCBP**

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following

menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormone replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

**Pregnancy**

WOCBP will undergo a pregnancy test at baseline and W16. The choice of test, urine or blood, will be determined by the sites, taking the accuracy and speed of processing in the local laboratory into account. In addition, WOCP will be instructed to contact their study physician immediately in the absence of menstruation or in case of other clinical evidence of pregnancy for further clarification by pregnancy testing. If, following initiation of the investigational product, it is subsequently discovered that a study patient is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued.

Any pregnancy withing the treatment period will be treated as AE and reported to the Sponsor within 24 hours of the site’s awareness of the event. A special AE-Pregnancy form will be completed. Protocol-required procedures for study discontinuation and follow-up must be performed on the patient unless contraindicated by pregnancy. Other appropriate pregnancy follow-up procedures should be considered if indicated. The sponsor of the study will initiate a follow-up of the patient upon conclusion of the pregnancy.

**Contraception**

**Highly effective methods for contraception**

Methods that can achieve a failure rate of less than 1% per year when used consistently and

correctly are considered as highly effective birth control methods.

Such methods include:

1. combined (estrogen and progestogen containing) hormonal contraception associated with

inhibition of ovulation.

o oral

o intravaginal

o transdermal

1. progestogen-only hormonal contraception associated with inhibition of ovulation:

o oral

o injectable

o implantable

1. intrauterine device (IUD)
2. intrauterine hormone-releasing system
3. bilateral tubal occlusion
4. vasectomised partner (Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the woman of childbearing potential trial participant and that the vasectomised partner has received medical assessment of the surgical success)
5. sexual abstinence (sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments)

**Limitations**

The study is a double blind RCT study, as such there are considerable measures to control the bias in place. Thus, the impact of the expected bias is judged as neglectable (see section 11.2). Owing to CMR-based inclusion criteria using standardised imaging method, we expect the results to be reliable and generalizable.

Recruitment might be a risk factor, if many participants do not show cardiac abnormalities in CMR, which is an inclusion criterion for the study. We have chosen the inclusion criteria to mitigate this risk. We have further calculated and planned for SCR failures. We have also chosen centres with a long-standing expertise in Cardiac Imaging, inflammatory cardiac conditions, and rheumatology e.g., Frankfurt am Main, Vienna, Kiel) and high flow of COVID-19 participants (Vienna, Greifswald) to minimize the risk of recruitment problems.

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